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Relative Stalk Sugar Yields Among Maize Populations, Cultivars, and Hybrids[1]

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ABSTRACT

Recent emphasis on alternate energy sources has prompted research on several crops as carbohydrate sources for conversion to fuel energy. We have been assessing maize (*Zea mays* L.) stalks as a potential sugar source for conversion to alcohol fuel. Two methods of determining sugar yields and their relationships with plant height and maturity were also evaluated. Fifteen open-pollinated maize cultivars or populations and 19 hybrids were evaluated for fermentable stalk sugar yields in replicated randomized complete block experiments in 1980 and 1981. The range of average sugar yields for the populations and cultivars was 23 to 55 g plant^{-1} when determinations were made on the basis of percent soluble solids by water content and 24 to 64 g plant^{-1} when determinations were based on high performance liquid chromatography analysis for fermentable sugars in the stalks. Comparable ranges for the hybrids were 25 to 50 g plant^{-1} and 17 to 52 g plant^{-1} by the respective methods. Hybrid means determined by the two methods were significantly correlated, while the methods for population and cultivar means were not significantly correlated because of the greater genetic heterogeneity within cultivars and populations compared to hybrids. Hybrid and cultivar or population 1980 means were highly correlated with those of 1981. The average sugar yields of cultivars and populations on a per plant basis were comparable to the hybrids, and large differences within both groups suggested that selection progress for improved yield is a reasonable expectation. The better performing entries of each group would be expected to produce sugar yields in excess of 3.5 Mg ha^{-1} at populations of 60 000 to 70 000 plants ha^{-1}.

Additional index words: ***Zea mays* L., Biomass production, Energy conversion, Fermentable sugars, HPLC, Refractometer readings, Sucrose.**

RECENT concerns about the cost and availability of energy in this country have resulted in efforts to identify and develop alternate sources of fuel for energy. The use of crop plants as a carbohydrate source for conversion to alcohol is one alternative being considered. Blackshaw (1912) indicated that maize (*Zea mays* L.) stalks had been considered as a source for sugar, cellulose, and alcohol since before the turn of the century. Clark, in 1913, emphasized the importance of preventing pollination, or ear removal, as an effective way of increasing the sugar content of maize stalk juice.

Interest was renewed during the 1940s in maize stalks as a source of fermentable sugars. Gore (1947) reported alcohol yields ranging from 477 to 1010 L ha^{-1} for sweet and field corn grown in New York. Singleton (1948) followed with a report that identified C103 as an inbred having higher sucrose and total sugar content than several others tested. Van Reen and Singleton (1952) later reported total sugar percentages in stalk juices exceeding 16% when samples were collected at 5 weeks after pollination. They confirmed earlier reports that plants with nonpollinated ears produced stalk juices having higher sugar concentrations. The nodes at ear height and above usually produced juice with higher sugar content. Hume and Campbell (1972) reported soluble solids concentration in the stalks was unaffected by plant density, but that a rapid decline in accumulated soluble solids occurred in pollinated plants during the grain-fill period. Total soluble solids in the stalks of plants increased until the end of the growing season for nonpollinated ears.

Singleton (1948) suggested hybrids with improved sugar concentrations in the stalk juices might be developed. Blanco et al. (1957) selected for high stalk sugar among plants from which grain had been harvested about 5 weeks earlier. They reported that selection for high sugar content was effective and that it was accompanied by an increase in grain protein percentage. When sampled at whole plant moisture percentages between 60 and 70, Cunningham et al. (1980) found that stalks of hybrids selected for high stalk sugar had sugar contents 1.2 to 1.6 times higher than those of the check hybrids. A recent evaluation by D'Ayala Valva et al. (1980) of maize stalk juices from 10 cultivars gave alcohol production estimates approaching 2000 L ha^{-1} for the best cultivars tested. Hills et al. (1983) compared one maize cultivar, one sweet sorghum (*Sorghum bicolor* L.) cultivar, and two beet (*Beta vulgaris* L.) cultivars for yields of fermentable carbohydrates converted to alcohol production. The maize yielded 4410 L ha^{-1} of alcohol and after appropriate adjustments for production costs, it was concluded that the crop cultivars tested had no great advantage over one another.

Objectives of the study were: 1) to evaluate several hybrids, populations, and cultivars of maize for their differences in production of fermentable carbohydrates, 2) to compare two methods of calculating relative sugar yields, one based on percent soluble solids of the stalk juice, the other on high performance liquid chromatography (HPLC) analysis.

MATERIALS AND METHODS

The plant materials used in these studies were chosen to represent a rather broad array of genotypes within each respective category of hybrids, cultivars, or populations. Having little information on their potential for sugar production, the selection of materials was based on diversity of origin.

Nineteen hybrids were planted in a 10-replicate, randomized complete block design, on 6 May 1980 and 3 April 1981. Single row plots 6.1 m long with 91 cm and 76 cm row spacing in 1980 and 1981, respectively, were grown at a plant density of approximately 50 000 ha^{-1}. Eleven sib-pollinated populations and four cultivars were planted on 23 April 1980 and 9 April 1981. The cultivars were stan-

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dard open-pollinated types, and populations included bulked collections and groups of common origin or bulked crosses of common collections with Corn Belt material. All populations and cultivars have been maintained by bulk-sibbing. The design and plot size were identical to the hybrid experiment except that only five replications were planted for the populations and cultivars in 1981.

The same sampling and harvest procedures were used for all tests. Ear shoots were bagged as they appeared in 1980 to prevent pollination of sampled plants, but a few ears were pollinated because of silk growth out from under shoot bags and bags removed by wind. In 1981, the easier, surer method was used of removing shoots from the plants within 5 days of silking. Any bias in sugar yield estimates due to change in procedure was assumed to be constant for all entries, since relative yields were our major interest. The internode below the ear was sampled from three plants in each plot at 8 weeks after anthesis. A portion (ca. 1 cm in length) of each stalk section (internode) was used to obtain a sample for the hand-held refractometer reading (Brix or percent soluble solids) by squeezing one to four drops of juice with pliers. The remaining portion of the three stalk sections from each plot was placed together and frozen immediately to be analyzed for sugars at the National Agricultural Energy Center (NAEC) in Peoria, IL. Ten additional plants from each plot were harvested at about 8 cm above the soil and weighed at 8 weeks after anthesis. Plant moisture percentage was obtained from a three-stalk sample by oven drying at 60°C and using weight differences to calculate water content and actual dry weight yields.

Sugar yields were calculated in grams of sugar per plant, by two methods: 1) water contents of samples from each plot were estimated as plant moisture percentage by total plot weight. Assuming that the average refractometer reading (percent soluble solids) for stalk juice samples from a plot were proportional to the sugar percentage of the juice yields, the relative sugar yields were calculated by multiplication of water content by percent soluble solids (PSS); 2) total dry weights were multiplied by the percentages of fermentable sugar (sucrose plus invert sugars) present on a dry weight basis, as determined by HPLC. The simple, inexpensive PSS estimates are easily obtained and would expedite selection progress if they could be used in place of more precise estimates, such as those obtained by HPLC. All errors in estimating relative sugar yields were assumed either to be offsetting or consistent in direction so that comparisons among genotypes remained meaningful.

Frozen samples were packed in dry ice for shipment to NAEC. For the HPLC analyses, frozen stalk sections received at NAEC were split lengthwise and then freeze-dried in a VirTis[5] sublimator (Model 100-SRC, VirTis Company, Inc.). Subsequently, these dried sections, containing about 1% moisture, were ground in a Thomas-Wiley[5] mill (Model ED-5) with a sieve containing 1-mm diameter perforations. A representative 2.0-g sample (dry solids basis) was shaken 2 h, at room temperature, with 25 ml of 50% ethanol (v/v). The mixture was then centrifuged 10 min at 10 000 rpm. The supernatant was subsequently analyzed for sugar (glucose, fructose, and sucrose) content by HPLC using a BioRad HPX-42[5] size-exclusion column and water for the mobile phase. Preliminary analyses determined that these procedures were sufficient to remove essentially all sugars from the stalk samples and that no significant amount of any sugars other than glucose, fructose, and sucrose were in the extracts.

[5] Mention of a commercial product does not constitute endorsement by USDA.

Table 1. Fermentable sugar yields of four corn cultivars and 11 populations grown at Tifton, GA in 1980 and 1981 as determined by two methods of analysis.†

	Method of obtaining fermentable sugar yields					
	Percent soluble solids			HPLC analysis		
Cultivar or population	1980	1981	1980–1981	1980	1981	1980–1981
	g plant^{-1}					
Nuevo Leon group	50	64	55	34	20	30
Vera Cruz crosses	45	53	48	54	38	49
Knightin 8-row	40	54	44	51	33	45
Robyn	40	50	43	66	61	64
Trinidad crosses	37	53	42	39	31	36
Eto crosses	40	45	42	44	21	36
Homedale	38	49	42	47	39	44
Alapaha	33	56	40	52	30	44
Antigua crosses	28	50	36	54	29	46
Cuba crosses	31	43	35	55	27	46
AES 801 group	33	32	33	38	22	32
Grenada crosses	23	45	30	30	12	24
Hawaii crosses	27	35	30	33	32	32
Puerto rico crosses	25	38	29	56	21	44
Zapalote Grande crosses	22	25	23	41	18	33
$LSD_{0.01}$	11.7	16.7	10.7	24.7	28.0	18.6
Mean	34	46	38	46	29	40

† Fermentable sugars refer to sucrose plus the invert sugars, fructose and glucose.

Sugar production, whether based on PSS or HPLC analysis, was converted to grams of fermentable sugar per plant for comparison of the two methods. Plot values were subjected to analysis of variance and means were compared by LSD.

RESULTS AND DISCUSSION

Wide variation existed among the cultivars and populations for fermentable sugar yields (Table 1). This variation suggests that substantial genetic variation also exists for stalk sugar content. Differences between average sugar yields of 1980 and 1981 were highly significant within the PSS and HPLC analysis methods. However, highly significant correlations of $r = 0.73$ and $r = 0.66$ between yearly cultivar and population means for sugar content within the PSS and HPLC analysis methods, respectively, demonstrate some consistency in the ranking of test entries from year to year.

The highest sugar yields among hybrids in 1980-1981 were two and three times as large as the lowest when measured by the PSS and HPLC analysis methods, respectively (Table 2). Correlations between 1980 and 1981 hybrid entry means were highly significant; $r = 0.73$ for the PSS method, and $r = 0.80$ for the HPLC analysis method.

Very little difference appears to exist between the hybrids and cultivars or populations as groups with respect to total stalk sugar yield on a per plant basis (Table 1 vs. Table 2). A large amount of variation does exist within each group, and hybrid yields would probably be greater on a land area basis since most of those hybrids tested will withstand substantially higher plant populations than the unimproved, nonhybrid materials. At populations of 60 000 to 70 000 plants ha^{-1}, sugar yields in excess of 3.5 Mg ha^{-1} could be expected for several of the better performing hybrids in our tests.

Table 2. Fermentable sugar yields for 19 commercial corn hybrids grown at Tifton, GA in 1980 and 1981 as determined by two methods of analysis.†

	Method of obtaining fermentable sugar yields					
	Percent soluble solids			HPLC analysis		
Hybrid	1980	1981	1980–1981	1980	1981	1980–1981
	g plant^{-1}					
Pioneer X5802	51	49	50	54	32	43
Coker 77	48	48	48	53	40	46
McNair X300	41	55	48	63	41	52
McCurdy 67-14	42	54	48	52	38	45
Pioneer X4816-A	46	45	45	54	30	42
Pioneer X304-C	46	44	45	41	26	34
DeKalb XL395	42	44	43	51	35	43
Dixie 18	36	48	42	38	31	34
DeKalb 1214	31	47	39	44	38	41
DeKalb XL80A	35	39	37	63	31	47
Coker 16	33	39	36	57	34	45
Ring Around RA2601	37	35	36	46	27	37
McNair 508	31	41	36	40	32	36
Pioneer 3030	33	36	34	33	22	28
Asgrow 101W	27	41	34	24	20	22
Funk G4507	27	37	32	17	18	17
Cargill HS50A	26	31	29	35	26	30
Pioneer 3369A	23	34	28	40	25	33
Sokota Sugar Chop	22	29	25	36	18	27
$LSD_{0.01}$	11.3	14.8	9.3	18.3	13.6	11.4
Mean	36	42	39	44	30	37

† Fermentable sugars refer to sucrose plus the invert sugars, fructose and glucose.

The range of values for entry means was only slightly larger for sugar by the HPLC analysis method of yield determination than for the PSS method. Considerable change in the ranking of cultivars and populations occurred for the two methods, however, as illustrated by nonsignificant correlations ($r < 0.30$) between yearly or 1980-1981 entry means of the two methods. Analogous correlations for hybrid means were highly significant ($r > 0.57$) indicating a greater consistency between methods among hybrids. The difference between methods among cultivars and populations might best be demonstrated by the fact that the best performing entry using PSS (Nuevo Leon Group = 55 g plant^{-1}) and the best performing entry using HPLC (Robyn = 64 g plant^{-1}) were both significantly different from one another within methods.

The correlation ($r = 0.69$) between 1980-1981 hybrid means of the two methods of determination was highly significant while 1980 and 1981 determinations within methods also had significant correlations at $r = 0.73$ for the PSS method and $r = 0.80$ for the HPLC analysis method. The reason for a significant correlation between methods for hybrid means and not for cultivar means may be related to maturity and plant height, which are much more uniform among hybrids than among cultivars. The correlation between maturity and PSS values ($r = 0.73$) that exists for cultivar and population means is a nonsignificant $r = 0.37$ for hybrids and those between PSS values and plant height are $r = 0.85$ and $r = 0.52$ for cultivars and populations, and hybrids, respectively.

Mean sugar yields per plant were significantly higher in 1981 (46 g) than in 1980 (34 g) when PSS values were used for the determination, while the reverse was true for values of sugar from HPLC analysis. The changes from one year to the next within methods may have been due to the late planting date, heat, and/or reduced rainfall in 1980, but it did not greatly influence the separation of hybrid means for either method. The 1980-1981 average hybrid yields per plant obtained for both methods were essentially equal (39 g vs. 37 g).

Summarizing, similar variation was observed for both methods of determining fermentable sugar yields and a relationship emerged between these yields and the plant height or maturity of populations and cultivars. Hybrids were more uniformly ranked by both methods of determination than were the cultivars and populations. The PSS method may not be as effective as the more precise HPLC method for use in a selection program, especially when plant materials of different maturity and size are used to form the breeding populations.

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